Assessment of IL-10 and IL-12 level among certain group of acute toxoplasmosis infections in Babylon aborted women.

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تقييم معيار الانترلوكين العاشر والثاني عشر في مجاميع من نساء بابل المجهضات جراء العدى الحادة بداء المقوسات الكوندية

الخلاصة

تم إجراء هذه الدراسة على أمصال ٢٤ امرأة أجهضن جراء العدوى الحادة بالمقوسات الكوندية والآتي أظهرن معيارا عاليا للغلوبيولين المناعي المضاد للمقوسات الكوندية نوع (M)، إضافة إلى أمصال عشرة من النساء الأصحاء كضابط للدراسة.

تم معايرة مستويات الغلوبيولينات المناعية والانترلوكينات باستخدام تقنية فحص ألـELISA (تقدير الامتزاز المناعي للانزيم المرتبط) والتي أجريت في مختبر الصحة العامة في بابل للفترة الممتدة من شهر كانون الثاني وحتى حزيران من عام(٢٠١٠).

وقد عكست هذه الدراسة بان مجموعة النساء المجهضات باعمارمن ٢٥ – ٣٢ سنة هن الأكثر عرضة للعدوى من بقية المجاميع ، وأظهرن الدراسة بدرجة قناعة عالية تغيرا في مستوى كلا الانترلوكينين (IL-10 و IL-12) في أمصال مجموعة النساء المجهضات لأول مرة .

كما أظهرت النتائج وبدرجة قناعة عالية أيضا تغيرا في مستويات كلا الانترلوكينين (IL-10 وIL-12) واللذان انخفضا في أمصال جميع مجاميع النساء المجهضات جراء العدوى والآتي اظهرن أجمعهن مستويات عالية من الغلوبيولين المناعي المضاد للمقوسات الكونيدية نوع M مقارنة بأمصال مجموعة ضابط الدراسة (السيطرة).

واستنتج من الدراسة انه بالإمكان مراقبة فعالية العدوى من خلال المعرفة للمستويات العالية للغلوبيولين المناعي المضاد للمقوسات الكوندية والانخفاض في مستوى كلا الانترلوكينين(IL-10 و IL-12) على التعاقب.

Abstract:

This study was carried out on 24 subjects infected with toxoplasmosis who had high levels of IgM (anti-*Toxoplasma gondii* antibodie), as well as 10 healthy people as a control matching age group. The *Toxoplasma gondii* immunoglobulin levels and both interleukines were detected by ELISA techniques, which were carried out in Babylon public health laboratory during the period from January till June 2010.

This Study reflected that, the main age group of infection ranged from 25-34 yeares old, with highly significant changes of both interleukins among first abortion toxoplasmosis patients.

The results also showed a highly significant change of both interleukins (IL-10 & IL-12) which are reduced among all age groups of patiens in association with high levels of anti-*T. gondii* IgM, in comparison with control samples. Monitoring of the activity of the disease is associated with high level of anti-*T. gondii* IgM and reduced level of both IL-10 and IL-12 respectively.

Introduction

T.gondii is a coccidian protozoan of world wide distribution that infects a wide range of vertebrates. It is one of the most common parasitic infections causing life-threatening encephalitis in the immunocompromised and congenital infection in newborns (Carolyn F.D. *et. al*, 1999). There are three routes of transmission. The first is the ingestion of infected animal tissues, the second route of infection is oocysts shed in cat feces; they can remain in this inert state for a long period of time and only come out of it when they are consumed by the appropriate organism; the third route of infection is transplacental, this is when the baby becomes infected through its mother's placenta. (Jason *et. al* 2009) The organism in human produces either congenital or postnatal toxoplasmosis, congenital infection which develops only when non immune mothers and infected during pregnancy. Fatal infection may develop in patients with AIDS, varying degree of diseases may occur in immuno-suppressed individual , resulting in retinitis or chorioretinitis, encephalitis, pneumonitis or various other condition ((Geo, F.A 2009)).

Cytokines such as IFN – γ and α (which activate macrophages function) are important for controlling tachyzoite replication during both acute and chronic phases of infection, IL-10 and IL-12 appear to be crucial at the initial phase of infection and less important during chronic toxoplasmosis. IL-12 is clearly important in initiating a strong and effective cell - mediated immunity against *T. Gondii* tachyzoite, IL-10 appears to modulate both IL-12 and IFN – γ synthesis in *vivo*, avoiding an excessive immune response that could cause extensive inflammation and host tissue damage. IL-10 and IL-12 are two major antagonist involved in regulating IFN- γ synthesis during the initial phases of infection. (Butcher *et. al*, 2005).

IL-12 appears to play an important, but transitory, role in protection against acute infection with *T. gondii* in the normal murine host (Khan *et. al*, 1994). Interleukins (IL-10, IL-12) and interferon gamma (IFN-γ) are major cytokines involved in the immune response against *T. gondii*. Nevertheless, the role of IL-10 and IL -12 was studded well by Estran, *et. al*, 2006, he had been report that IL- 10 and IL-12 have no effect on penetration, replication, or cystogenesis of the *T. gondii* .

The objective of this study was directed to find if there was any relation between both interleukins IL -10 and IL – 12 and *T. gondii* infection in correlation with number of abortion in Babylon aborted women.

Materials and Methods:

Twenty four out of 165 aborted women sera sample were collected from aborted women who had history of repeated abortion and had high titer of anti – *T. gondii* IgM enrolling in Babylon public health laboratory, in period extended from January to June 2010. Also 10 apparently healthy individual sera sample were used as a control. Sera sample were classified according to the number of abortion of each subject into three groups (once, two or three abortion).

Blood samples were collected aseptically in sterile plain tubes, in a volume of 5 ml by vein puncture for each subject. After clotting sera samples were separated in small aliquots and labeled separately and storied at (-20 ċ) till used by ELISA test for estimation of anti – *T. gondii* immunoglobulin (IgM) and measurement of both IL -10 and IL – 12 values. ELISA test were performed according to restriction manuals of the manufacturer Biosource Company. A quantitative kits supplied by this company was used to calculate the result and give an appropriate concentration by making standard

curve (Biosource company, Nivelles – Belgium). IL – 10 and IL - 12 levels were measured in all collected sera sample including control one.

Results:

All sera sample were tested by ELISA test and anti -*T. gondii* IgM, as well as interleukins IL -10 and IL – 12 were quantitatively measured as shown in table (1). The main value of IL – 10 was 14.8 pg / ml whereas the main value for IL – 12 were 434.58 pg / ml in comparison with 55.6 pg /ml and 799 pg / ml for controls of both interleukins respectively. There was marked decrease in the levels of both interleukins in comparison with control one. The table also reflected high value for IgM in aborted women sera.

Measurement of IL – 10 in the first aborted women group revealed a marked decrease value which was (20 pg / ml) in comparison with control sera sample which was (55.6 pg / ml) this indicated that control value 2.6 time more than first aborted women group value. The results reflected also low IL – 12 levels (633 pg / ml) as compared with control one (table2).

On the other hand second abortion women group sera reflected marked decrease of both interleukins (IL – 10 and IL – 12) which was 4.6 time ,and 2.8 time lower than control samples value for both interleukins respectively, consequently the value of both interleukins was 1.7 and 2.1 time lower than first abortion women group respectively table (3).

However both interleukins was decreased also in third abortion women group when compared with control sera values and reach a level lower than those of the other first and second abortion groups. In addition to that there was much more clear reduction in IL-10 than IL – 12 and directly proportional to increase number of abortion, table (4) and (5). The distribution of aborted women groups in correlation with age range shown in table(6), (25 - 34 years) old aborted women group reflected a reduced level of both cytokines (IL-10 and IL-12) as well as significant anti-*T.gondii* IgM level, this result lead us to a suggestion that this age is the optimum age group for reproduction, and complication of pregnancies and abortion due to *T. gondii* is one of several factors causing abortion at any age group of pregnant women.

Table (1) shows the interleukins level among all groups of aborted women in correlation with anti - T. gondii IgM - titer .

Statistical data		IL - 12 pg /ml		IL- 10 pg /ml		Anti-*T.gondii* (IgM) Level
		Test	Control	Test	Control	Test
Mean		434.58	799	14.8	55.6	47.9
No.*		24	10	24	10	24
SD**		250.4	374.3	12	15.2	8.81
SE***		51.1	18.3	4.82	2.46	1.79
t-test	(*) P. value	0.002		0.000		0.000
	Sign.	H.S @		H.S		H.S

*=number of samples tested, ** = standard deviation, *** = standard error, @ = highly significant. (*) =Probability Value.

Table (2) interleukin level among toxoplasmosis patients in correlation with sequences of abortion (first abortion).

Statistical data		IL- 12 pg /ml		IL- 10 pg /ml	
		First abortion	Control	First abortion	Control
Mean		603	799	20.7	55.6
No.		10	10	10	10
SD		306	374.3	17.3	15.2
SE		9.5	18.3	5.45	4.82
t- test	P. value	0.18		0.01	
	Sign.	N.S		H.S	

Table (3) interleukin level among toxoplasmosis patients in correlation with sequences of abortion ((second abortion)) .

Statistical data		IL- 12 pg /ml		IL- 10 pg /ml	
		Second abortion	Control	Second abortion	Control
Mean		289.5	799	12.1	55.6
No.		8	10	8	10
SD		60.4	374.3	2.19	15.2
SE		21.38	18.3	0.77	4.82
t-test	P. value	0.008		0.004	
	Sign.	H.S		H.S	

Table (4) interleukin level among Toxoplasmosis patients in correlation with sequences of abortion ((Third abortion)) .

Statistical data		IL- 12 pg /ml		IL- 10 pg /ml	
		Third abortion	Control	Third abortion	Control
Mean		297	799	9.71	55.6
No.		6	10	6	10
SD		663.2	374.3	1.56	15.2
SE		21.3	18.3	0.64	4.82
t- test	P. value	0.003		0.000	
	Sign.	H.S		H.S	

Table (5) interleukin level among toxoplasmosis patients in correlation with sequences of abortion (first, second and third abortion).

Statistical data		IL- 12 pg /ml		IL- 10 pg /ml		IL- 12 pg /ml		IL- 10 pg /ml		IL- 12 pg /ml		IL- 10 pg /ml	
		First abortion	Control	First abortion	Control	second abortion	Control	second abortion	Control	Third abortion	Control	Third abortion	Control
Mean		603	799	20.7	55.6	289.5	799	12.1	55.6	297	799	9.71	55.6
No.		10	10	10	10	8	10	8	10	6	10	6	10
SD		306	374.3	17.3	15.2	60.4	374.3	2.19	15.2	663.2	374.3	1.56	15.2
SE		9.5	18.3	5.45	4.8	21.3	18.3	0.77	4.82	21.3	18.3	0.64	4.82
t-test	P. value	0.18		0.01		0.008		0.004		0.003		0.000	
	Sign.	N.S.		H.S.		H.S.		H.S.		H.S.		H.S.	

Table (6) result of interleukins according to age group among the toxoplasmosis Babylon aborted women.

Age group (years)	No. of subject	IL-12	IL-10	Anti-*T.gondi* IgM Level
15-24	4	297	10.9	47.9
25-34	15	438	13.9	45.3
35- 44	2	683	11.5	43.4
45-54	3	347	17.1	46.7

Discussion:

Maternal infection with a large number of different organisms has been associated with an increased risk of miscarriage spontaneous abortion. Fetal or placental infection by the offending organism then leads to pregnancy loss. Examples of infections that have been associated with miscarriage include infections by *Listeria monocytogenes, T. gondii, parvovirus B19, rubella, herpes simplex, cytomegalovirus, and lymphocytic choriomeningitis virus* (wikipedia.). Some acquired immunity may develop in the cause of infection. Antibodies titers in mother, as detected in either blood or milk, tend to fall within a few months, prenatal infection is limited to infects bone of mothers who where first exposed during their pregnancy. Immune deficiency disease, immunosuppressant drugs, or change in host resistance may cause chronic infection (Eric *et. al*, 1998).

False-positive results were obtained using the IgG enzyme immunoassay (EIA). During pregnancy, positive results may falsely reassure, and patients should be tested for *Toxoplasma*-specific IgM to differentiate between current infection and immunity.

Interleukins (IL-10, IL-12) and interferon (IFN-γ) are major cytokines involved in the immune response against *T. gondii*. Nevertheless, the role of IL-10 and IL -12 was studded well by Estran *et. al*, 2006 he had been report that IL- 10 and IL-12 have no effect on penetration, replication, or cystogenesis of the *T. gondii*. However there correlation with abortion recurrence is not studded till now . CD 19 B- cells and CD8+ T cells steadily increase for 8 days after infection , CD8 + T cell were rapidly recruited to the site of infection and increased faster than CD4 + T cells , associated with development of long – lasting immunity to *T. gondii* ((Jasen *et. al* 2009)).

It had been improved that *T. gondii* had a suppressive effect on macrophage pro inflammatory cytokine production(Butcher *et. al*, 2005). Protective immunity against *T. gondii* is mediated by the host cellular immune response, IL-12 is clearly important in initiating a strong and effective CMI against *T. gondii* tachyzoite. Interleukin-12 stimulates NK cells to produce gamma interferon (IFN-γ), which is able to enhance host protection against this parasite. IL-12 appears to play an important, but transitory role in protection against acute infection with *T. gondii* in the normal murine host (Khan *et. al*, 1994), this finding inconsistent with present results in aborted women groups. The parasite rapidly induces production of the type 1 promoting cytokines (IL-12) are then activated and triggered to synthesize (IFN – γ). The major mediator of host resistance during acute and chronic phase of infection ((Khan and Kasper, 1996)). During the acute phase, a concomitant IL-10 response dampens the systemic (type 1) cytokine production and prevent lethal immunopathology (Elia D Tait; Christopher A Hunter 2009).

Cytokine are more critical for protective immunity in both during the acute and chronic phases of *T. Gondii* infections *(Yap and Sher, 1999).* IL-10 appears to modulate both IL-12 and IFN – γ synthesis *in vivo*, avoiding an excessive immune response that could cause extensive inflammation and host tissue damage (Yap and Sher, 1999) . This may explain the main cause of fetal damage and consequently fetal abortion. General look for our results gave an indication that IL – 10 was much more inhibited by *T. gondii* infection.

Table (1) show a highly significant (H.S) reduction of IL-10 and IL- 12 in comparison with control sample as well as with high level of anti-*T. gondii* IgM . This revealed that there might be rapid consumption of this cytokines at acute stage of this disease and consequently lowering their levels. Table (2) show no significant (N.S) difference in IL-12, and highly significant in (IL-10)among women group of first abortion, this result lead to a suggestion that the parasite might have a little effect on(IL-12)production where as their inhibiting effect mainly directed toward T helper cell and reduced production of (IL-10). Cell mediated immune response are essential for host control of intracellular infection with *T. gondii*. IL-10 limits infection-induced immune pathology, it is characterized by its ability to antagonize (Th1) responses (Fiorentino *et. al*, 1989). Today, IL-10 is considered to be an inhibitor of Th1, Th2 and Th17 immune responses (Moore *et. al*, 2001, Lieberman & Hunter, 2002; O'Garra & Vieira, 2007). It is produced by macrophages, monocytes, DC, B cells, and CD4+ and CD8+ T cells, and acts broadly on accessory cells to down-regulate proinflammatory cytokine production and major histocompatibility complex (MHC) and costimulatory molecule expression. Thus, T cell priming and activation are dampened primarily through IL-10's effects on accessory cells, although there are reports that IL-10 may also directly suppress CD4+ T cell proliferation and cytokine production (Couper *et. al*, 2008).

Table (3) show H.S. reduction in both interleukins among women group of second abortion, this might be duo to insufficiency CMI especially macrophage and T h cell. However table(4) shown H.S. reduced of both cytokines among women group with third abortion, this result revealed that no difference in parasite activity at both second and third abortion and the infection might be prolonged for long time and may lead to chronic state rather than acute phase disease or suggesting that a latent infection persistency at theses group aborted women .Anyhow table (5) shows IL-12 and IL-10 values among three aborted women groups in comparison with control group. Furthermore the age group of 25-34 years old representing the highest number of aborted women ,who shown reduced level of both cytokines (IL-10 and IL12) as well as significant anti –*T. gondii* IgM level, this might be due to the fact that this age representing optimum age group for reproduction, whereas aborted women group of 15-24 years old reflected the highest mean of anti- *T. gondii* IgM level, and the lower IL-10 level, table (6).

We concluded that *T. Gondii* can be expected as a causative agent of abortion indirectly by monitoring the level of both IL-10 and IL-12 which is indicated by its reductive values especially IL-10. Decreased level of IL-10 rather than IL-12 among the first aborted patients might be refer to the activity of humeral immunity against this parasite rather than the cellular immunity in association with the higher anti-*Toxoplasam* IgM level. We recommended that large scale study need for evaluation of the importance of both interleukin, by using mice model through the evaluation of therapeutic use of these interleukins in pregnant mice after experimental infection with this protozoan . Decreased levels of both interleukins during second and third abortion may refer to the

increased exposure to the parasite or there is a latent infection stimulated within time of pregnancy leading to repeated abortion and high level of IgM against *Toxoplasma* infection.

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